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RAPID *IN VITRO* PROPAGATION OF *ANANAS COMOSUS VAR. MICROSTACHYS* USING CROWN EXPLANTS

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ABSTRACT

The present study was conducted from December 2023 to August 2024 at the Department of Biotechnology, Ramniranjan Jhunjhunwala College, Mumbai, to develop an efficient in vitro propagation protocol for *Ananas comosus var. microstachys*. Crown explants were disinfected and cultured on Murashige and Skoog (MS) medium with varying concentrations of 6-benzylaminopurine (BAP) and kinetin (KN) (0.25 to 2.0 $mg.L^{-1}$) for shoot induction, and indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) (0.5 to 3.0 $mg.L^{-1}$) for rooting. The highest shoot proliferation was observed at 2.0 $mg.L^{-1}$ BAP, with 25 shoots/explant in the first subculture and 38 in the second. The maximum shoot length reached 5.5 cm. Rooting was optimal on full-strength MS medium supplemented with 1.0 $mg.L^{-1}$ IBA, yielding 10.67 roots/shoot with an average length of 7 cm. Primary hardening in a 1:1 cocopeat and vermiculite mixture resulted in 98% survival, while soil and sand (1:1) proved suitable for secondary hardening. The protocol ensures rapid, large-scale multiplication of dwarf pineapple for commercial floriculture applications.

Key words: BAP, Crown, Dwarf pineapple (*Ananas comosus var. microstachy*s), Hardening

INTRODUCTION

Floriculture, or flower farming as it is popularly called, is a discipline of horticulture that deals with study of growing and commercialising flowers, cut flowers, foliage, and ornamental plants (Wani *et al.*, 2018). Floriculture makes use of flowering and attractive plants for gardens and their use as raw materials in the pharmaceutical, cosmetic, and perfume industries (Sankari *et al.*,2020). The floral and ornamental plant market is characterised by its dynamic nature, always seeking new offerings.

Ananas comosus var. *microstachys* is the most economically and commercially important plant in the family *Bromeliaceae* (Nashima *et al.*, 2015) that has adapted to land or epiphytic habitats and endures extreme climates with shade or full sunlight (Sharma *et al.*, 2024). Dwarf pineapple is cultivated around the world for its ornamental properties and is now gaining a lot of demand (Hilo De Souza *et al.*, 2014) in Asian countries for its novelty and extended shelf life as a cut flower. Thus, due to the rarity of this plant, its monetary value is very high, and its planting material is in great demand.

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This perennial monocot features a central stem topped with an inflorescence and fruit, with the fruit sporting a crown (Benzing, 2023). Beneath every leaf axil of a pineapple crown is a dormant axillary bud from which new shoots, called slips, can develop and after fruiting, axillary buds may grow into suckers, producing a ratoon crop (Sulaiman *et al.*, 2020). However, natural propagation through structures like crowns is slow, posing a challenge for breeders aiming to efficiently boost selected clones.

All the conventional methods used to propagate the ornamental pineapple require a long time to produce a plant and then to flower. The conventional propagation technique gives rise to only one plant and thus is insufficient for mass production. Hence, a propagation technique that can produce several plants from a single vegetative part would be of great value. Thus, micro-propagation can be used as it allows rapid, efficient, and mass cultivation of plants. This was the first time to report *in vitro* multiplication of ornamental pineapple through the use of the crown as an explant. While several studies have successfully utilized crown explants for pineapple micropropagation in different varieties, including 'cv. Josapine' where crown explants achieved 88% shoot induction with 2.0 mg/L BAP (Sriskanda *et al*., 2025), the present study represents the first comprehensive protocol specifically optimized for *Ananas comosus var. microstachys* using crown explants. Previous pineapple tissue culture studies predominantly focused on commercial varieties such as MD2 (Sulaiman *et al.*, 2020) (Guzmán-Antonio *et al.*, 2023) and 'Smooth Cayenne' (Lakho *et al*., 2023), employing various explant types including shoot tips, nodal segments, and sucker buds (Reinhardt *et al.,* 2018). However, the ornamental dwarf pineapple variety *microstachys* (Fig.1) has received limited attention in micropropagation research despite its growing demand in floriculture. In the present study, a simple protocol was developed to propagate dwarf pineapple through tissue culture methods from the crown explants to ensure an abundant supply of this plant material for commercial cultivation.

Fig. 1. Dwarf pineapple *Ananas comosus* var. *microstachys*

MATERIAL AND METHODS

The mother plants were obtained from a nursery in Karjat, Maharashtra. The fruit stalks were harvested from the plants, and subsequently, the crowns were bilaterally excised from them and were used as explants (Zulkarnain *et al.*, 2018). These crowns were then washed under running water to remove all the traces of soil present on them and were further used to establish an aseptic culture.

The explants underwent a thorough washing process with tap water, followed by the addition of 1% of Bacillocid special. Subsequently, they were surface sterilised using 70% ethanol followed by 0.1% mercuric chloride solution for 15 minutes and rinsed four times with sterile double-distilled water within the Laminar Air flow chamber. Small crown segments (Fig.2) measuring 0.5-1.0 cm were placed on a modified MS medium supplemented with specific concentrations of growth regulators (BAP, KN) either individually or in combination, along with 3000 $mg \cdot L^{-1}$of sucrose and 0.7% agar. The pH of the medium was set to 5.7 using 0.1N HCl before autoclaving at 121°C for 20 minutes. The cultures were then kept at 25 ± 2°C with a 16-hour photoperiod. Subculturing was performed at 21-day intervals. The proliferated shoots were sub-cultured again to induce multiple

Fig. 2. Crown explant

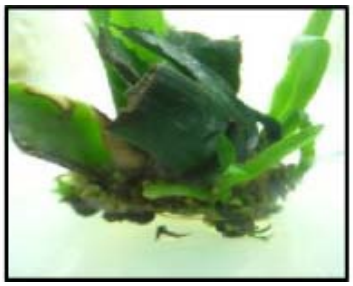

Fig. 3. Shoot bud proliferation

Fig. 4. *In vitro* rooting

shoot bud formation (Fig 3). The regenerated multiple shoots were divided, and individual shoots were transferred to a modified MS medium with varying concentrations of IBA and IAA for root induction (Fig 4) (Siposova *et al.*, 2021). Controls consisting of hormone-free MS basal medium were included in all experiments to verify that explants remained viable but did not exhibit callus induction, shoot proliferation, or rooting in the absence of exogenous growth regulators.

Requirements of cytokinin for *in vitro* shoot induction

The effect of cytokinin on explants for shoot induction was studied by inoculating them on a modified Murashige and Skoog medium supplemented with BAP and KN at concentrations ranging from 1.0 to 5.0 $mg.L^{-1}$. The crowns thus sterilised were transferred into the medium with various concentrations of cytokinin for the production of *in vitro* shoots.

The cytokinin (0.5–2.0 $mg.L^{-1}$ BAP and KN) and auxin (0.5-3.0 $mg.L^{-1}$ IBA and IAA) concentration ranges were selected based on previous reports demonstrating optimal *in vitro* responses in pineapple. BAP at 2.0 $mg.L^{-1}$ has repeatedly produced high shoot multiplication, including 16.7 shoots per explant in cv. Smooth Cayenne (Lakho *et al*., 2023).and 6.85 shoots per explant in cv. Josapine, when combined with 0.5 $mg.L^{-1}$ NAA (Sriskanda *et al*., 2025). For rooting, IBA (1.0–2.0 $mg.L^{-1}$) is consistently superior to other auxins, with 1.0 $mg.L^{-1}$ IBA yielding more roots and greater root length than other auxins (Lakho *et al*., 2023).

Requirement of auxin for *in vitro* callus induction

Explants were inoculated on the modified Murashige and Skoog's medium supplemented with auxins like NAA and 2,4-D. Each of them was tested with concentrations, viz- 0.5, 1.0, 1.5, 2.0 $mg.L^{-1}$. The responses of the explants were recorded at an interval of 7 days.

Synergistic effect of auxin and cytokinin for callus induction

The optimum concentration of cytokinins like BAP, Kinetin and auxins like NAA, 2,4-D was combined to test the synergistic effects of these on the explants' response for callus induction and further regeneration.

Rooting of *in vitro-derived* plants

The *in vitro* regenerated shoots were separated individually and sub cultured on full-strength and half-strength modified MS medium supplemented with 1 $gm.L^{-1}$ activated charcoal and 0.5 to 2.0 $mg.L^{-1}$ of IBA, NAA and IAA, respectively.

Vertically cut crowns callused approximately three weeks after the explants were placed on callus induction medium. The explants callused on MS medium supplemented with 0.5 $mg.L^{-1}$ NAA + 1.0 $mg.L^{-1}$ BAP gave the best result. The callus, initiated on media supplemented with the above hormonal combinations, was compact. The proliferated

Table 1. Synergistic effect of cytokinin + auxin on the callus-generating ability of the crown of *A. comosus* var *microstachys*

Concentration of plant Growth regulator BAP + NAA (mg.L^{-1})	Number of explants tested	Callus response expressed in percentage	Duration in days for callus initiation
0.0+0.0	12	-	-
0.5+0.0	12	10	26
0.5+0.5	12	25	26
1.0+0.5	12	92	18
1.5+1	12	33	22
2.0+1	12	66	23
0.0+0.5	12	-	-

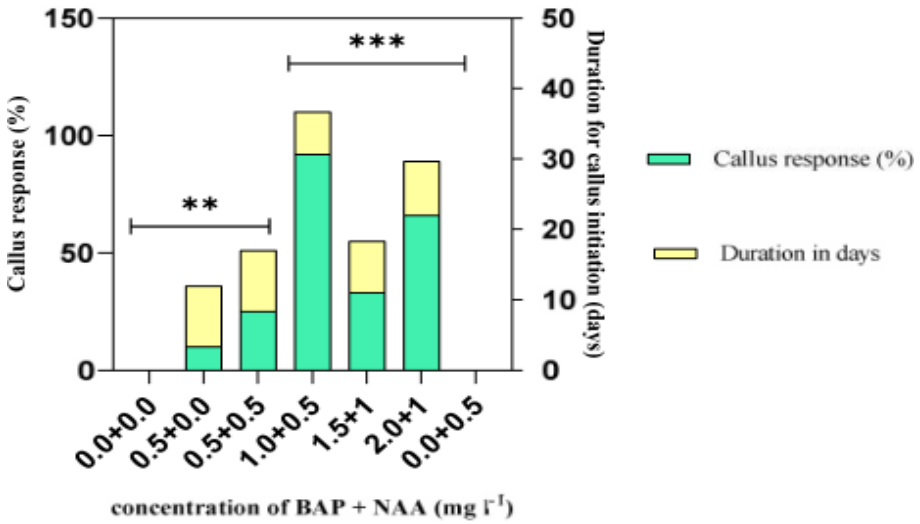

Fig 5. Synergistic effects of different concentrations of BAP and NAA on callus-generating ability of the crown of *A. comosus* var *microstachys*.

Callus response (%) (green bars, left y axis) and duration required for callus initiation (days) (yellow bars, right y axis) are shown for explants cultured on media supplemented with the indicated combinations of BAP and NAA (x axis). Data were analyzed using two way ANOVA followed by post hoc multiple comparison tests. The relationship between callus response and induction time was evaluated using Spearman correlation. Error bars represent 95% confidence intervals calculated from 12 replicates per treatment, and statistical significance is indicated by asterisks (**p < 0.01; ***p < 0.001).

Calli were sub-cultured into cytokinin-containing medium for shoot initiation.

For shoot induction callus, it was subcultured on the regeneration medium. The results show that the highest number of shoots was found to regenerate in the medium fortified with 2.0 mg.L^{-1} BAP alone. The BAP-NAA combination was not so efficient in initiating shoot regeneration from callus.

Table 2.Effect of BAP cytokinin on the shoot-generating ability of the callus.

Concentration of plant Growth regulator BAP (mg.L^{-1})	Number of explants tested	Shoot response in percentage	Number of shoots/explants.		
			7Days	14 Days	21Days
0.0	7	0	-	-	-
0.5	7	20	1	3	3
1.0	7	40	3	6	8
1.5	7	60	15	22	30
2.0	7	60	25	32	38

For rooting of excised shoots, either a single or a combination of two or three auxins was used routinely. In the present experiment, 1.0 mg.L^{-1} IBA was found to be the optimal concentration for root induction. Root formation was not observed when shoots were cultured on a medium lacking auxin. Other auxins tested were IAA and NAA, whose results were insignificant, as the roots developed were very few and not healthy.

RESULTS AND DISCUSSION

Establishment of explants

Establishment of aseptic culture was the most crucial stage of the *in vitro* culture of ornamental pineapple. The primary issue encountered was contamination, which was attributed to the presence of both endogenous and exogenous microflora inherited from the explants. To overcome this problem, a severe

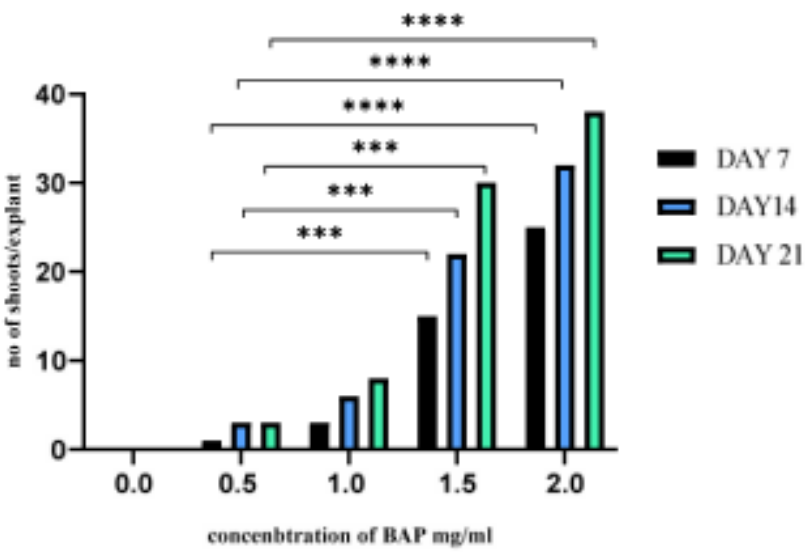

Fig 6. Effect of BAP concentration and culture duration on shoot proliferation.

The number of shoots per explant was recorded on day 7 (black bars), day 14 (blue bars), and day 21 (green bars) for explants cultured on media containing the indicated BAP concentrations (mg/ml) along the x axis. Data were analysed using two way ANOVA followed by Dunnett's multiple comparison test. Statistical significance is indicated by asterisks (***p < 0.001; ****p < 0.0001).

Table 3. Effect of auxin on root generating ability on the shoot of *Ananas comosus* var *microstachys.*Observation on 20 days after subculture.

Concentration of plant Growth regulator IBA (mg.L^{-1})	Number of shoots tested for rooting	Percentage root response	Average number of roots	Average length of roots(cm)
0.0	7	-	-	-
0.5	7	-	-	-
1.0	7	100	10	7
1.5	7	40	4	3
2.0	7	60	3	4

sterilisation protocol had to be followed. Even then, with the best of the technique applied, it was found that the survival rate of the explants in the laboratory condition was 80 per cent.

Another problem was browning of the media due to the abundance of polyphenols and their oxidation; this could be controlled by immersing the explants in water containing 1% (w/v) ascorbic acid or citric acid. Alternatively, in the initial stages of the culture, the explants were sub cultured more frequently to prevent the browning of the explants.

Synergistic effect of auxin and cytokinin

BAP and NAA in various combinations were added to Murashige and Skoog's medium for their ability to induce callus and shoots. These combinations of BAP and NAA gave good results for callus induction in crowns as compared to auxin 2,4-D, which was also used for the same purpose. Although all the combinations tried gave results but vertically cut crowns callused approximately three weeks after the explants were placed on callus induction medium. The explants callused on MS

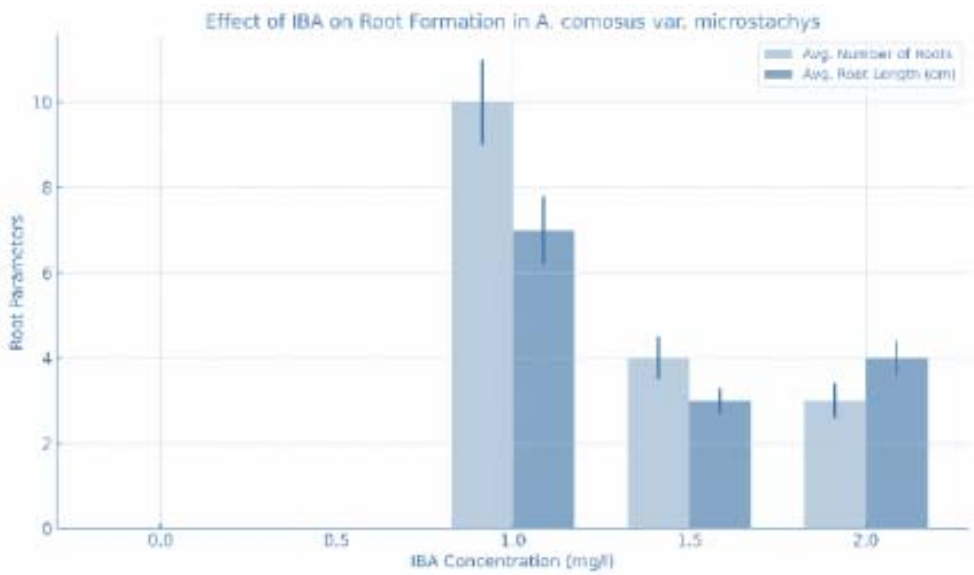

Fig 7. Effect of auxin on root generating ability on the shoot of *A. comosus var microstachys.* Observation on 20 days after subculture.

medium supplemented with 1.0 mg.L^{-1} BAP+0.5 mg.L^{-1} NAA gave the best result. The callus initiated on media supplemented with the above hormonal combinations was compact. The proliferated calli were sub-cultured into cytokinin-containing medium for shoot initiation. To check that it was only in combination of BAP and NAA that resulted in callus induction, the singular effect of BAP and NAA was also tried for callus induction, but neither of them gave results. Though only NAA was found to be necrotic, BAP did induce shoots in some explants at concentrations higher than that of NAA.

Effect of cytokinin on shoot regeneration

Benzyl amino purine was the most effective cytokinin in terms of multiple shoot induction. Percentage responses of the explants were also high compared to other cytokinins. BAP concentrations ranging from 1.0mg.L^{-1} to 5.0mg.L^{-1} were tested; almost all concentrations showed multiple shoot formation, but at a concentration of 2.0mg.L^{-1}, the shoot formation rate was maximum; it was around 25 shoots per explant within a week's incubation. Moreover, the plants obtained were also healthy. It was concluded that a concentration of 2.0mg.L^{-1} BAP was best; therefore, further experiments were continued with the same concentration. Kinetin was also used for the induction of shoots, but was not found to be as effective as compared to BAP.

Effect of 2,4-D on callus induction

Effects of various concentrations of 2,4-D were observed on ornamental pineapple, although most of the concentrations were found to be necrotic, callusing was observed in some explants inoculated in media containing 1.0 mg.L^{-1} 2,4-D after 30 days of subculture. This callus became brown later and did not proliferate.

Effect of various concentrations of IBA, IAA and NAA on root regeneration

For rooting of excised shoots, several concentrations of IBA, IAA and NAA were used. In the rooting experiment, Murashige and Skoog's medium with 1.0 mg.L^{-1} IBA was found to be the best for root induction. This concentration of IBA resulted in the greatest average number of roots, which was 10 andthe average length was found to be 7 cm. Root formation was not observed when shoots were cultured on a medium lacking auxin. Compared to IBA, IAA and NAA were also supplemented respectively into MS medium to check their effect on root initiation in the *in vitro* grown shoots. But IBA gave a better response to root initiation.

Hardening of *in vitro* derived plants

Acclimatisation is the final but necessary step in all plant propagation schemes. Here, plants have to adapt to new environmental conditions such as low relative humidity, higher light intensity and fluctuations of temperature and stress (Rambabu *et al.*, 2021). Hardening of the *in vitro-derived* plantlets was done in a greenhouse in small pots. It was found that in the primary hardening, 1:1 vermiculite and coco peat were very effective. The survival rate of the plantlets was 98%. In the secondary hardening, soil and sand were used in the ratio of 1:1, which also proved to be a good medium for hardening.

CONCLUSION

The present study successfully established a simple, reproducible, and efficient in vitro micropropagation protocol for dwarf ornamental pineapple using crown explants. The combination of 1.0 mg.L^{-1} BAP and 0.5 mg.L^{-1} NAA produced the best callus induction, while 2.0 mg.L^{-1}BAP proved most effective for shoot multiplication. Rooting was optimally achieved with 1.0 mg.L^{-1} IBA, and the survival rate of hardened plantlets reached 98 per cent. This protocol can serve as a reliable tool for mass multiplication of *Ananas comosus var.microstachys* to meet commercial floriculture demands.

REFERENCES

Benzing, D. H. 2023. *Bromeliaceae:* A brief profile and some topics that warrant further inquiry. Selbyana, 34, 1–79.

Guzmán-Antonio, A., Canto-Flick, A., Aviles-Viñas, S. 2023. Highly efficient system for the micropropagation and acclimatization of pineapple in vitro plants (*Ananas comosus* L. Merr, var. MD2). Journal of Applied Botany and Food Quality*, 96*, 109–116. https://doi.org/10.5073/JABFQ.2023.096.014

Hilo De Souza, E., De Carvalho Costa, M. A. P., Santos-Serejo, J. A., Vidigal, F. and De Souza, D. 2014. Selection and use recommendations in hybrids of ornamental pineapple. Revista Ciência Agronômica, 45(2), 409–416.

Lakho MA, Jatoi MA, Solangi N, Abul-Soad AA, Qazi MA and Abdi G. 2023. Optimizing in vitro nutrient and ex vitro soil mediums-driven responses for multiplication, rooting and acclimatization of pineapple. Sci Rep., Jan 23;13(1):1275. https://doi.org/10.1038/s41598-023-28359-9

Rambabu, M., Ujjwala, D. and Ramaswamy, N. 2021. Hardening and acclimatisation of an endangered forest tree, *Givotia rottleriformis* Griff. Journal of the Indian Botanical Society*, 101 (1-2), 158-162.*https://doi.org/10.5958/2455-7218.2021.00014.0

Reinhardt, D. H. R., Bartholomew, D. P. and Souza, F. V. D. 2018. Advances in pineapple plant propagation. Revista Brasileira de Fruticultura, 40(6), e-302.https://doi.org/10.1590/0100-29452018302

Sankari, A., Loganayaki, P., Kayalvizhi, K., Kavitha, M. and Jerlin, R. 2020. Standardisation of planting materials in tuberose (*Polianthes tuberosa* L.) cv. Arka Prajwal. International Journal of Current Microbiology and Applied Sciences, 9(1), 2046–2053. https://doi.org/10.20546/ijcmas.2020.901.232

Sharma, A., Kumar, L., Malhotra, M., Singh, A. P. and Singh, A. P. 2024. *Ananas comosus* (pineapple): A comprehensive review of its medicinal properties, phytochemical composition, and pharmacological activities. Journal of Drug Delivery and Therapeutics, 14(5), 148–157. https://doi.org/10.22270/jddt.v14i5.6557

Šípošová, K., Labancová, E., Kuèerová, D., Kollárová, K. and Vivodová, Z. 2021. Effects of exogenous application of indole-3-butyric acid on maize plants cultivated in the presence or absence of cadmium. Plants, 10(11), Article 2355. https://doi.org/10.3390/plants10112355

Sriskanda, D., Khor, S. P., Tan, L. V., Subramaniam, S., Isa, N. M. and Chew, B. L. 2025. In vitro Shoot Regeneration from Crown and Sucker Explants of *Ananas comosus cv.* Josapine. Tropical Life Sciences Research, *36*(3), 135-155.https://doi.org/10.21315/tlsr2025.36.3.7

Sulaiman, S., Yusuf, N. A. and Awal, A. 2020. Effect of plant growth regulators on in vitro culture of pineapple (*Ananas comosus* L. Merr) MD2 variety. Food Research, 4(Suppl. 1), 110–114. https://doi.org/10.26656/fr.2017.4(S5).017

Wani, M. A., Nazki, I. T., Din, A., Iqbal, S., Wani, S. A., Khan, F. U. and Neelofar. 2018. Floriculture sustainability initiative: The dawn of a new era. Floriculture Today, 91–127. https://doi.org/10.1007/978-3-319-75190-0_4

Zulkarnain, Neliyati andEliyanti. 2018. Plantlet regeneration from crown bud slicing of pineapple (*Ananas comosus*). Biosaintifika: Journal of Biology & Biology Education, 10(3), 484–490.

Golwalkar,S.Sucheta, Shah,V. Ichha and Khan,Y. 2025. Rapid in vitro propagation of A*nanas comosus var. Microstachys* using crown explants. The Journal of Research ANGRAU,53 (4): 14-21.